

Convergent evolution of bright coloration and toxicity in frogs

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Brightly colored organisms abound in nature, and the evolutionary forces underlying this profusion of color have been the subject of scientific interest ever since Darwin. Aposematism, where color and pattern serve as advertisements of unprofitability to predators, is one of the main mechanisms thought to generate selection in favor of bright coloration and contrasting pattern. The theoretical underpinnings of aposematism, particularly the mechanisms whereby new bright mutants become established in a population, remain the subject of considerable interest (1). On an empirical level, a basic prediction is that brightly colored organisms will be unpalatable. Although many brightly colored organisms have proven to be toxic (2), the hypothesis of aposematism further predicts that the evolution of toxicity will occur before, or in tandem with, the evolution of bright coloration. Testing this prediction requires information on the evolutionary relationships of the species involved. Few studies have concomitantly analyzed the historical trajectories of both coloration and toxicity, thereby identifying the evolutionary sequence and association of both traits (3). In this issue of PNAS, Santos *et al.* (4) use phylogenetic analysis to investigate the evolution of coloration and toxicity in poison frogs of the family Dendrobatidae.

The poison frogs of South America and Central America (toxic members of the family Dendrobatidae) provide a well known example of bright coloration associated with toxicity (in this case, the presence of lipophilic alkaloids in the skin) (5). The dendrobatids are a species-rich taxon (>200 species) with both brightly colored and cryptic members (Fig. 1). Traditionally, the brightly colored species were thought to form a monophyletic group, mainly on the basis of shared toxins (6). Most cryptic species were placed in the genus *Colostethus* although no shared derived characteristics united them (7). *Colostethus* has now been divided into several genera, but their phylogenetic relationships remain poorly understood (7). Previous molecular phylogenetic analyses have focused on the colorful dendrobatids by using a few species of *Colostethus* as outgroups (e.g., ref. 8). Santos *et al.* (4) present a hypothesis of



Fig. 1. Two members of the Neotropical frog family Dendrobatidae. (Left) *Colostethus trilineatus*. All members of this genus are cryptically colored. (Right) *Epipedobates macero*, a brightly colored and toxic member of the family. Research reported in this issue demonstrates that bright coloration and toxicity have evolved together many times in this family of frogs.

evolutionary relationships (a phylogenetic tree) based on DNA sequence data that includes a broad sample of both colorful and cryptic dendrobatids. Their tree reveals that the brightly colored species are not monophyletic. Instead, some species of *Colostethus* are more closely related to brightly colored species than to other cryptic members of the family. Mapping the evolution of coloration on the phylogenetic tree identifies five separate occasions in which colorful species have arisen from cryptic ancestors.

Statistical Analyses

How robust is this finding? Using parametric bootstrapping to assess the statistical support for particular groups within their phylogenetic tree (9), Santos *et al.* (4) tested various alternatives to five origins of bright coloration. These tests provided support for at least four separate origins of bright coloration. Scenarios with three or fewer origins are significantly less likely to explain the data. On another front, a group of European and South American researchers recently found evidence for multiple origins of bright coloration in the dendrobatids by using DNA sequences from (for the most part) different species of *Colostethus* (10). The results of that study were not as robust as those reported by Santos *et al.* (4) (probably due to shorter lengths of the DNA sequences used to make inferences). Nevertheless, the fact that two different research groups, working with different species of *Colostethus*, have arrived at the same result solidifies the conclusion

that bright coloration evolved multiple times in the dendrobatid frogs.

These findings beg the question of whether bright coloration coevolved with toxicity in this group. The theory of aposematism predicts that bright coloration will evolve in response to the evolution of toxicity (1). In a phylogenetic framework, bright coloration should appear on the same internode (internal branch) of the tree as toxicity, or on a descendant internode or branch. Previous tests (11) of the prediction of coevolution between bright coloration and toxicity compared these traits as continuous characters within the toxic dendrobatids (including a few members of *Colostethus* as outgroups), by using a statistical technique known as comparative analysis of independent contrasts (12). This analysis revealed a significant relationship between levels of toxicity and the contrast of coloration to a leaf-littered background. The power of comparative analysis to detect associations between traits over the course of evolution depends on the number of independent evolutionary origins of the traits. These origins provide the statistically independent events used to investigate associations (12). The broader phylogenetic hypothesis presented by Santos *et al.* (4), with its expansion of the number of independent origins of bright coloration and toxicity, provides the opportunity to implement more powerful tests

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of the hypothesis of coevolution between toxicity and coloration. Using only discrete characters (e.g., colorful vs. cryptic, toxic vs. palatable) and a comparative method designed for such characters known as the concentrated changes test (13), Santos *et al.* (4) demonstrate a highly significant association between the evolution of toxicity and bright coloration. This result provides more robust support for the hypothesis that toxicity and bright coloration have coevolved in the dendrobatids. Future research should focus on sampling the toxicity of a broader array of *Colostethus* species. Research to date has not revealed the presence of lipophilic alkaloids in any *Colostethus* (14) although one species has been demonstrated to have water-soluble skin toxins (15). However, a limited number of species have been sampled, and it would be useful to garner more information on the toxicity of cryptic dendrobatids, to conduct more thorough tests of the hypothesis of aposematism.

Diet, Toxicity, and Coloration

Evidence from a variety of research suggests that many of the skin toxins of dendrobatid frogs are sequestered from dietary sources (arthropods) (16). Some researchers have questioned the validity of a comparative approach to the evolution of aposematism in the dendrobatids, given variation in toxin abundance and given that there is no information on the availability of dietary alkaloids in ancestral populations (16). However, if changes in the availability of toxins prevented bright coloration from accurately representing toxicity in ancestral populations for significant periods, then aposematism should not evolve, and we would not expect currently toxic populations to be brightly colored. The evidence provided by Santos *et al.* (4) and by previous research is consistent with the hypothesis that dietary toxins were available to the ancestors of brightly colored species and is not consistent with the alternative.

The phylogeny presented here offers an expanded basis on which to examine the association of diet with toxicity and coloration. Several researchers have proposed that dietary specialization, in terms of increased proportions of ants

and a consequent reduction in dietary breadth, is associated with the evolution of toxicity and coloration (e.g., ref. 17). Caldwell (17) proposed that, as non-toxic, cryptic dendrobatid frogs came to consume a higher proportion of ants, selection favored efficient systems of toxin removal, presumably through the skin. In turn, predation pressure would have favored frogs that retained toxins in their skin as a defense mechanism. The new phylogenetic hypothesis presented by Santos *et al.* (4) suggests that independent evolutionary associations between dietary specialization and toxicity have occurred three times although

The results demonstrate a highly significant association between the evolution of toxicity and bright coloration.

the authors note that more data are required for confirmation. On the other hand, the authors note that, in at least one case, toxicity and bright coloration have evolved in the absence of specialization on ants. Statistical tests of the evolutionary association between dietary specialization and toxicity must await dietary data on a wider array of species.

Aposematism and Mimicry

The study of aposematism is intimately entwined with the study of mimicry (18). Several researchers have suggested that mimicry occurs among the dendrobatids, between different brightly colored species (e.g., ref. 19). Efforts at testing these hypotheses have been severely hampered by a lack of information on the phylogenetic relationships among species. Recently, molecular phylogenetic analysis has revealed a mimetic radiation, in which a single species of poison frog has evolved to resemble three different species in different geo-

graphic regions (20). All of the species involved are toxic; hence, this study provides a rare example of Müllerian mimicry (in which toxic species evolve to resemble each other) in vertebrates. The expanded phylogeny presented by Santos *et al.* (4) offers the opportunity to investigate other cases that may involve mimicry among poison frogs. For example, *Epipedobates parvulus* and *Allobates* (formerly *Epipedobates*) *zaparo* both share a striking appearance, with granulated skin on the dorsum suffused with deep red. These largely sympatric species were thought to be closely related, and their shared color and pattern are thought to be due to recent common ancestry. This new phylogenetic hypothesis indicates that these two species are not at all closely related, indicating that they may share color and pattern through mimicry.[†]

This study highlights the importance of using multiple, independent characters in phylogenetic analyses. In earlier systematic studies, a lack of informative morphological characters meant that the presence of shared toxins was one of the only characteristics uniting the poison frogs (5). As Santos *et al.* (4) illustrate clearly, convergence can profoundly affect phylogenetic analyses when only a few characters are used to infer evolutionary relationships. Their study also highlights the importance of broad sampling of species in evaluating evolutionary relationships. The first molecular phylogenetic studies of dendrobatids included only limited numbers of cryptic species of *Colostethus* (e.g., ref. 7). The expanded species sampling used here has paid great dividends toward a better understanding of the phylogenetic relationships among dendrobatids. These results will, of course, incur substantial taxonomic revision for this family. Once accomplished, however, the taxonomy of the dendrobatid frogs will reflect their phylogenetic relationships rather than their toxicity or coloration.

[†]Santos, J. C., Coloma, L. A. & Cannatella, D. C., Forty-fifth Annual Meeting of the American Society of Ichthyologists and Herpetologists, July 3–8, 2002, Kansas City, MO, p. 265.

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